LISTING OF THE CLAIMS

This Listing of the Claims replaces all previous listings of the claims in this application.

1. (Original) A method for recombinantly expressing a mature Streptococcus pyogenes exotoxin B (SpeB) polypeptide in a host cell, the method comprising transforming, transducing, transfecting or infecting a host cell with a polycistronic plasmid, the plasmid comprising (a) a polynucleotide sequence encoding a SpeB pro-polypeptide domain and (b) a polynucleotide sequence encoding a mature SpeB polypeptide, and culturing the host cell under conditions which permit the expression of the mature SpeB polypeptide and the SpeB pro-polypeptide domain by the host cell, and wherein the mature SpeB polypeptide is soluble in the host cell.

2. (Original) The method of claim 1, wherein the SpeB pro-polypeptide domain is further defined as a polypeptide comprising amino acid residues 28 through 145 of SEQ ID NO:2.

3. (Original) The method of claim 1, wherein the mature SpeB polypeptide is further defined as a polypeptide comprising amino acid residues 146 through 398 of SEQ ID NO:2.

4. (Currently amended) The method of claim 1, wherein the mature SpeB polypeptide is further defined as a polypeptide comprising amino acid residues 146 through 398 of SEQ ID NO:2 wherein the cysteine at amino acid residue 192 ~~of the mature SpeB polypeptide~~ is substituted by a serine.

5. (Original) The method of claim 1, wherein the mature SpeB polypeptide is immunogenic in a mammalian host.

6. (Original) The method of claim 1, wherein an antibody specific for the mature SpeB polypeptide cross-reacts with a wild-type SpeB polypeptide and neutralizes SpeB polypeptide activity.

7. (Original) The method of claim 1, wherein the plasmid is a T7 promoter-containing plasmid.

8. (Currently amended) The method of claim 7, wherein the plasmid is selected from the group consisting of pET, pRSET, pCRT7-CTTOPO, [[and]] pIVeX, and mixtures thereof.

9. (Original) The method of claim 1, wherein the host cell is a bacterial cell.

10. (Original) The method of claim 9, wherein the host cell is E. coli.

11. (Currently amended) The method of claim 10, wherein the E. coli is a strain selected from the group consisting of BLR(DE3), BLR(DE3)pLysS, AD494(DE3), AD494(DE3)pLysS, BL21(DE3), BL21(DE3) pLysS, BL21(DE3)pLysE, BL21(DE3)pLacI, BL21trxB(DE3), BL21trxB(DE3)pLysS, HMS174(DE3), HMS174(DE3)pLysS, HMS174(DE3)pLysE, Origami(DE3), Origami(DE3)pLysS, Origami(DE3)pLysE, Origami(DE3)pLacI, OrigamiB(DE3), OrigamiB(DE3)pLysS, OrigamiB(DE3)pLysE, OrigamiB(DE3)pLacI, Rosetta(DE3), Rosetta(DE3)pLysS, Rosetta(DE3)pLysE, Rosetta(DE3)pLacI, Tuner(DE3), Tuner(DE3)pLysS, [[and]] Tuner(DE3)pLacI, and mixtures thereof.

12. (Original) A method for recombinantly expressing a mature SpeB polypeptide in a host cell, the method comprising: (a) transforming, transducing, transfecting or infecting a host cell with (i) a plasmid comprising a polynucleotide sequence encoding a SpeB pro-polypeptide domain and (ii) a plasmid comprising a polynucleotide sequence encoding a mature SpeB polypeptide; and (b) culturing the host cell under conditions suitable to co-express the SpeB pro-polypeptide domain and the mature SpeB polypeptide, wherein the mature SpeB polypeptide is soluble in the host cell.

13. (Original) The method of claim 12, wherein the SpeB pro-polypeptide domain is further defined as a polypeptide comprising amino acid residues 28 through 145 of SEQ ID NO:2.

14. (Original) The method of claim 12, wherein the mature SpeB polypeptide is further defined as a polypeptide comprising amino acid residues 146 through 398 of SEQ ID NO:2.

15. (Currently amended) The method of claim 12, wherein the mature SpeB polypeptide is further defined as a polypeptide comprising amino acid residues 146 through 398 of SEQ ID NO:2 wherein the cysteine at amino acid residue 192 ~~of the mature SpeB polypeptide~~ is substituted by a serine.

16. (Original) The method of claim 12, wherein the mature SpeB polypeptide is immunogenic in a mammalian host.

17. (Original) The method of claim 12, wherein an antibody specific for the mature SpeB polypeptide cross-reacts with a wild-type SpeB polypeptide and neutralizes SpeB polypeptide activity.

18. (Original) The method of claim 12, wherein the plasmid is a T7 promoter-containing plasmid.

19. (Currently amended) The method of claim 18, wherein the plasmid is selected from the group consisting of pET, pRSET, pCRT7-CTTOPO, [[and]] pIVeX, and mixtures thereof.

20. (Original) The method of claim 12, wherein the host cell is a bacterial cell.

21. (Currently amended) The method of claim 20, wherein the host cell is E. coli [[col]].

22. (Currently amended) The method of claim 21, wherein the E. coli is a strain selected from the group consisting of BLR(DE3), BLR(DE3)pLysS, AD494(DE3), AD494(DE3)pLysS, BL21(DE3), BL21(DE3) pLysS, BL21(DE3)pLysE, BL21(DE3)pLacI, BL21trxB(DE3), BL21trxB(DE3)pLysS, HMS174(DE3), HMS174(DE3)pLysS, HMS174(DE3)pLysE, Origami(DE3), Origami(DE3)pLysS, Origami(DE3)pLysE, Origami(DE3)pLacI, OrigamiB(DE3), OrigamiB(DE3)pLysS, OrigamiB(DE3)pLysE, OrigamiB(DE3)pLacI, Rosetta(DE3), Rosetta(DE3)pLysS, Rosetta(DE3)pLysE, Rosetta(DE3)pLacI, Tuner(DE3), Tuner(DE3)pLysS, [[and]] Tuner(DE3)pLacI, and mixtures thereof.

23. (Withdrawn) A method for producing a mature SpeB polypeptide comprising the steps of: (a) recombinantly expressing in a host cell a plasmid comprising a

polynucleotide sequence encoding a mature SpeB polypeptide, wherein the SpeB polypeptide forms an insoluble polypeptide aggregate in the host cell; (b) solubilizing the polypeptide aggregate, wherein the solubilized polypeptide is defined as a non-native mature SpeB polypeptide; (c) refolding the non-native mature SpeB polypeptide in the presence of one or more chaperone proteins, wherein the non-native mature SpeB polypeptide is folded into a native mature SpeB polypeptide; and (d) recovering the native mature SpeB polypeptide.

24. (Withdrawn—Currently Amended) The method of claim 23, wherein the one or more chaperone proteins are selected from the group consisting of GroEL, ~~GroEUGroES~~ GroEL/GroES, peptidyl-prolyl isomerase (PPI), peptide disulfide isomerase (PDI) and a SpeB pro-polypeptide domain.

25. (Withdrawn) The method of claim 23, wherein the chaperone protein is a SpeB pro-polypeptide domain comprising amino acid residues 28 through 145 of SEQ ID NO:2.

26. (Withdrawn) The method of claim 23, wherein the mature SpeB is a polypeptide comprising amino acid residues 146 through 398 of SEQ ID NO:2.

27. (Withdrawn) The method of claim 26, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

28. (Withdrawn) The method of claim 23, wherein the insoluble polypeptide aggregate is further defined as an inclusion body.

29. (Withdrawn) The method of claim 23, wherein solubilizing the polypeptide is a denaturant selected from the group consisting of urea, guanidinium chloride and heat.

30. (Withdrawn) A method for recombinantly expressing a mature SpeB polypeptide in a host cell comprising expressing in a host cell a polycistronic plasmid comprising (i) a polynucleotide sequence encoding a mature SpeB polypeptide and (ii) a

polynucleotide sequence encoding a GroEL polypeptide, wherein the mature SpeB polypeptide is soluble in the host cell.

31. (Withdrawn) The method of claim 30, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

32. (Withdrawn) The method of claim 30, wherein the plasmid further comprises a polynucleotide encoding a GroES polypeptide.

33. (Withdrawn) A method for producing a mature SpeB polypeptide comprising the steps of: (a) transforming, transducing, transfecting or infecting a host cell with a polycistronic plasmid comprising (i) a polynucleotide sequence encoding a mature SpeB polypeptide and (ii) a polynucleotide sequence encoding a GroEL polypeptide; (b) culturing the host cell under conditions suitable to express the mature SpeB polypeptide and the GroEL polypeptide, wherein the mature SpeB polypeptide is soluble in the host cell; and (c) recovering the native mature SpeB polypeptide.

34. (Withdrawn) The method of claim 33, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

35. (Withdrawn) A mature SpeB polypeptide produced according to the method of claim 1.

36. (Withdrawn) A mature SpeB polypeptide produced according to the method of claim 12.

37. (Withdrawn) A mature SpeB polypeptide produced according to the method of claim 23.

38. (Withdrawn) A mature SpeB polypeptide produced according to the method of claim 30.

39. (Withdrawn) A mature SpeB polypeptide produced according to the method of claim 33.

40. (Withdrawn) An immunogenic composition comprising the SpeB polypeptide of claim 35.

41. (Withdrawn) An immunogenic composition comprising the SpeB polypeptide of claim 36.

42. (Withdrawn) An immunogenic composition comprising the SpeB polypeptide of claim 37.

43. (Withdrawn) An immunogenic composition comprising the SpeB polypeptide of claim 38.

44. (Withdrawn) An immunogenic composition comprising the SpeB polypeptide of claim 39.

45. (Withdrawn) A method of immunizing a mammalian subject against S. pyogenes comprising administering to the subject an immunogenic amount of the composition of claim 40.

46. (Withdrawn) A method of immunizing a mammalian subject against S. pyogenes comprising administering to the subject an immunogenic amount of the composition of claim 41.

47. (Withdrawn) A method of immunizing a mammalian subject against S. pyogenes comprising administering to the subject an immunogenic amount of the composition of claim 42.

48. (Withdrawn) A method of immunizing a mammalian subject against S. pyogenes comprising administering to the subject an immunogenic amount of the composition of claim 43.

49. (Withdrawn) A method of immunizing a mammalian subject against S. pyogenes comprising administering to the subject an immunogenic amount of the composition of claim 44.

50. (Withdrawn) A polycistronic plasmid comprising (a) a polynucleotide sequence encoding a SpeB pro-polypeptide domain and (b) a polynucleotide sequence encoding a mature SpeB polypeptide, wherein the mature SpeB polypeptide is soluble when expressed in a host cell.

51. (Withdrawn) The plasmid of claim 50, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

52. (Withdrawn) The plasmid of claim 50, wherein the plasmid is a T7 promoter-containing plasmid.

53. (Withdrawn) A plasmid comprising a polynucleotide sequence encoding a SpeB pro-polypeptide domain and a plasmid comprising a polynucleotide sequence encoding a mature SpeB polypeptide, wherein the mature SpeB polypeptide is soluble when expressed in a host cell.

54. (Withdrawn) The plasmid of claim 53, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

55. (Withdrawn) The plasmid of claim 53, wherein the plasmid is a T7 promoter-containing plasmid.

56. (Withdrawn) A polycistronic plasmid comprising (a) a polynucleotide sequence encoding a mature SpeB polypeptide and (b) a polynucleotide sequence encoding a GroEL polypeptide, wherein the mature SpeB polypeptide is soluble when expressed in a host cell.

57. (Withdrawn) The plasmid of claim 56, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

58. (Withdrawn) The plasmid of claim 56, wherein the plasmid is a T7 promoter-containing plasmid.

59. (Withdrawn) A polycistronic plasmid comprising (a) a polynucleotide sequence encoding a mature SpeB polypeptide, (b) a polynucleotide sequence encoding a GroEL polypeptide and (c) a polynucleotide sequence encoding a GroES polypeptide, wherein the mature SpeB polypeptide is soluble when expressed in a host cell.

60. (Withdrawn) The plasmid of claim 59, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

61. (Withdrawn) The plasmid of claim 59, wherein the plasmid is a T7 promoter-containing plasmid.

62. (Withdrawn) A polycistronic plasmid comprising (a) a polynucleotide sequence encoding a mature SpeB polypeptide and (b) a polynucleotide sequence encoding one or more polypeptides selected from the group consisting of GroEL, GroES, SpeB pro-polypeptide domain, PDI and PPI, wherein the mature SpeB polypeptide is soluble when expressed in a host cell.

63. (Withdrawn) The plasmid of claim 62, wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by a serine.

64. (Withdrawn) The plasmid of claim 62, wherein the plasmid is a T7 promoter-containing plasmid.

65. (Withdrawn) A host cell transformed, transduced, transfected or infected with the plasmid of claim 50.

66. (Withdrawn) A host cell transformed, transduced, transfected or infected with the plasmid of claim 53.

67. (Withdrawn) A host cell transformed, transduced, transfected or infected with the plasmid of claim 56.

68. (Withdrawn) A host cell transformed, transduced, transfected or infected with the plasmid of claim 59.

69. (Withdrawn) A host cell transformed, transduced, transfected or infected with the plasmid of claim 62.

70. (Withdrawn) The method of claim 24, wherein the PPI is a S. pyogenes RopA PPI.

71. (Withdrawn) The plasmid of claim 62, wherein the PPI is a S. pyogenes RopA PPI.

72. (New) The method of claim 10, wherein the E. coli strain is a derivative of BL21 or K-12.

73. (New) The method of claim 72, wherein the E. coli cell is a modified strain:
- (i) having reduced or absent Lon protease activity;
- (ii) having reduced or absent OmpT protease activity;
- (iii) having reduced or absent RecA activity;
- (iv) having reduced or absent thioredoxin reductase activity;
- (v) having reduced or absent glutathione reductase activity;
- (vi) expressing a T7 RNA polymerase gene under lacUV5 control;
- (vii) expressing T7 lysozyme;
- (viii) having reduced or absent LacY activity;
- (ix) expressing tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA;
- (x) or any combination thereof.

74. (New) The method of claim 21, wherein the E. coli strain is BL21, K-12, or mixtures thereof.

75. (New) The method of claim 74, wherein the E. coli cell is a modified strain:
- (i) having reduced or absent Lon protease activity;
- (ii) having reduced or absent OmpT protease activity;
- (iii) having reduced or absent RecA activity;
- (iv) having reduced or absent thioredoxin reductase activity;
- (v) having reduced or absent glutathione reductase activity;
- (vi) expressing a T7 RNA polymerase gene under lacUV5 control;

(vii) expressing T7 lysozyme;

(viii) having reduced or absent LacY activity;

(ix) expressing tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA;

(x) or any combination thereof.